An Observation of Somatic Chromosomes from Spider Embryo-cells

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Synopsis

MATSUMOTO, Seiji (Department of Biology, Pre-Medical Section, Kyorin University, Tokyo): An observation of somatic chrmosomes from spider embryo-cells. *Acta arachnol.*, **27** (Special number): 167-172 (1977).

Preparations of somatic chromosomes were made using spider embryo-cells by a flame-dry technique with a colchicine- and a hypotonic-treatment. The somatic chromosome numbers of 4 species of spiders studied were as follows: 24 and 26 in *Acusilas coccineus*, 26 and 28 in *Anyphaena pugil*, 28 and 30 in both *Hasarius doenitzi* and *Pardosa T-insignita*. Somatic chromosomal preparation made from embryo-cells of eggs are successful for observing the chromosomes of spiders. Especially, it is considered that the technique demonstrated in this paper is useful for examination of chromosomes of spiders which are too small for dissecting their gonads from them.

Hitherto, the microscopic preparations of the chromosomes of spiders have been made by the technique of the paraffin section and/or the squash method. The gonads of the spider, especially the testes, have been used as the material for making the chromosomal preparations.

In this work, an application of a flame-dry technique and the use of the spider eggs were tried to make the preparations of the somatic chromosomes. The somatic chromosomes of the spiders, *Acusilas coccineus, Anyphaena pugil, Hasarius doenitzi* and *Pardosa T-insignita*, were observed. Then the chromosome numbers examined were compared with those reported by the previous investigators, PAINTER (1914), HACKMAN (1948), SUZUKI (1954), BOLE-GOWDA (1958), and MITTAL (1964, 1966a, 1966b).

Materials and Methods

The following species of spiders were used: *Acusilas coccineus* SIMON, 1895, *Anyphaena pugil* KARSCH, 1879, *Hasarius doenitzi* KARSCH, 1879, and *Pardosa T-insignita*

(BÖSENBERG et STRAND, 1906). The eggs of 3 species except for those of *Hasarius doenitzi* were obtained from their egg-cocoons collected at our university campus. The eggs of *Hasarius doenitzi* were laid by mature females collected from the same area and then bred in vials.

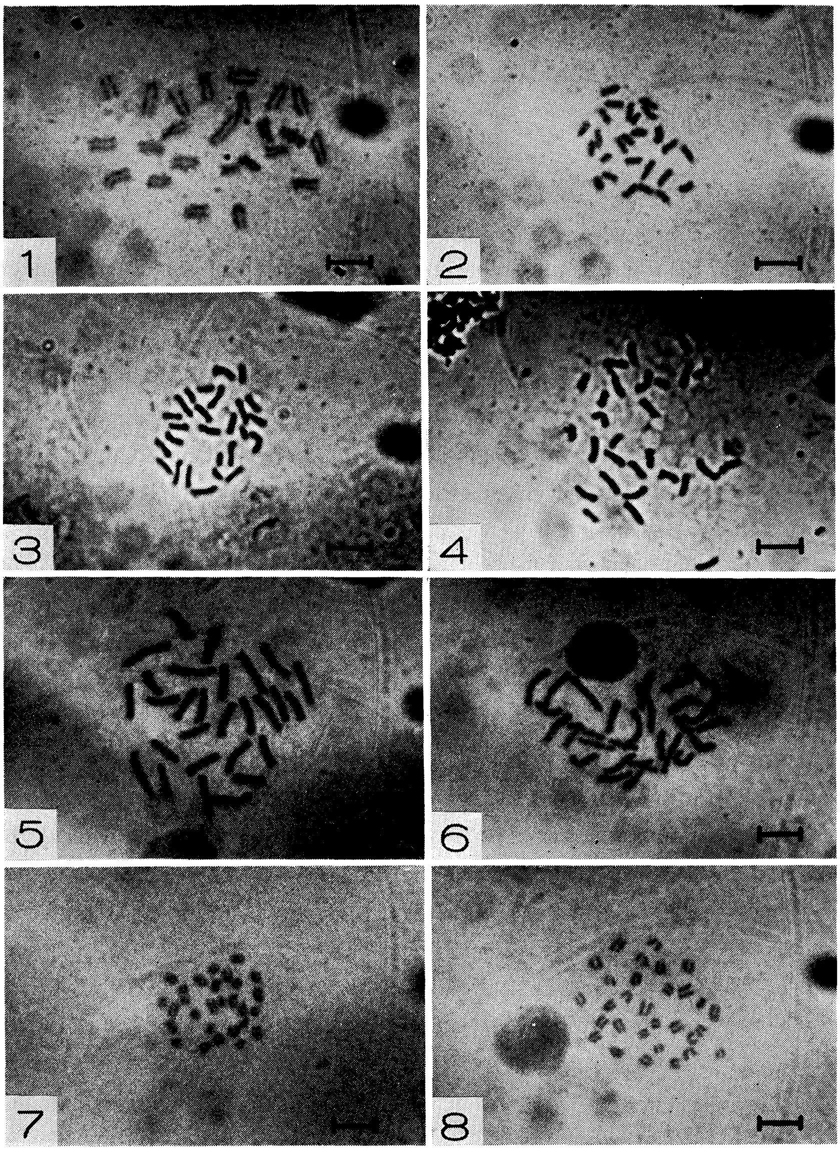
Processes of making preparations are as follows. A drop of a physiological salt solution is poured into a centrifuge tube. A single egg is soaked in the solution and crushed by a glass rod. As the physiological salt solution, 0.7 percent sodium chloride (pH 7.2) is used. A drop of 0.1 percent colchicine solution dissolved in the physiological salt solution is added to the embryo-cell suspension made from the crushed egg. After the colchicine solution is mixed in the embryo-cell suspension, the mixture is allowed to stand for 40 minutes. After 40 minutes, 2 drops of distilled water are added to the mixture in order to make a hypotonic condition. After 5 minutes, the embryo-cells are fixed by addition of 4 drops of fixing fluid, which is a mixture of glacial acetic acid (1 part) and absolute ethyl alcohol (3 parts). Thirty minutes later, the fixed embryo-cells are centrifuged at 1000 rpm for 5 minutes; the supernatant fluid is discarded from the centrifuge tube; 4 drops of new fixing fluid are added to the tube. The embryo-cells left on the bottom of the tube are suspended in the fixing fluid and centrifuged again under the same condition (1000 rpm, 5 minutes). The same operation as above, which consists of the exchange of the supernatant, the suspending and centrigufing of the embryo-cells, is carried out once again. In order to be made into a preparation, the suspension of the fixed and well-rinsed embryo-cells is dropped on a slide and flame-dried. An aceto-orcein is used as the staining solution. All the processes can be carried out at room temperature.

Results

Metaphase cells were observed from all microscopic preparations made from the embryos in various development stages. Preparations made from the spider embryos in the stage of appendage-formation had the maximum number of metaphase cells among

Table 1. Somatic chromosome numbers in the embryo-cells of 4 species of the spiders.

Species (Family)	Chromosome numbers
Acusilas coccineus (Araneidae)	24 & 26
Anyphaena pugil (Anyphaenidae)	26 & 28
Hasarius doenitzi (Salticidae)	28 & 30
Pardosa T-insignita (Lycosidae)	28 & 30



Figs. 1–8. Photographs of somatic chromosomes of the spiders. Figs. 1–2, 3–4, 5–6, and 7–8 show the somatic chromosomes from the embryo-cell of the spiders of *Acusilas coccineus, Anyphaena pugil, Hasarius doenitzi,* and *Pardosa T-insignita* respectively. The scale in the every figures indicates 10 micro.

preparations. Colchicine gives a different effect to the embryo-cells of each species of the spiders used. The metaphase chromosomes of the embryos of the spider, *Pardosa T-insignita*, were most strongly effected by colchicine.

Table 1 shows the somatic chromosome numbers of 4 species of the spider used in the present examination.

Discussion

In this work, the microscopic preparations of somatic chrmosomes from spider eggs were made by an application of a flame-dry technique. As a technique of tissue culture in spiders has not yet been contrived, spider embryos were used for making the preparation of the somatic chromosomes as materials. It is apparently the most imperfect point of this technique that the sex of the material cannot be determined directly. WYSOKI (1968) used eggs for his investigation of the chromosomes in phytoseiid mites. His technique, however, was not a drying method but a smear method. Although the sex of the egg cannot be determined from his preparation either, the sex-mechanism in the phytoseiid mite which was used by him and his coworkers is of the haplo-diploid type related to parthenogenesis (AMITAI, WYSOKI and SWIRSKI, 1969). The mechanism of the sex-determination in this mite is indeed different from that in the spider. When sex-determination is made through the examination of the chromosomal preparation made from the spider egg, the knowledges obtained by previous investigators should be referred enough.

In the result of the present work, the chromosome number of *Pardosa T-insignita* was seen to be equal to that reported by SUZUKI (1954). According to his result the sex-mechanism in this species is of the X_1X_2O-type, and the chromosome number of the germ cell in the male is either 13 (only 13 autosomes) or 15 (13 autosomes+2 sex-chromosomes). So the somatic cells have 28 chromosomes in the male and 30 chromosomes in the female. Of the other species studied with this technique, the followings were considered: the chromosome number of the germ cell in the male is $n=11$ or 13 in *Acusilas coccineus*, $n=12$ or 14 in *Anyphaena pugil,* and $n=13$ or 15 in *Hasarius doenitzi*. It is also considered that the mechanism of sex-determination is of the XXO-type in every species.

Three species (*Araneus dumetorum*, *A. ventricosus* and *Neoscona scylla*) of the familay Araneidae are exceptions which have different numbers of chromosomes from those of the majority of the araneids (HACKMAN, 1948; SUZUKI, 1954; MITTAL, 1966b). It is interesting that two of these three exceptions, i.e. *A. ventricosus* and *N. scylla*, are found in Japan.

Both species of *Anyphaena accentuata* and *A. saltibunda* have been studied karyologically. The chromosome number of *A. pugil* is the same as that of *A. accentuata* but not that of *A. saltibunda* (PAINTER, 1914; HACKMAN, 1948).

According to MITTAL (1964), the chromosome numbers of 33 species in the family Salticidae have already been investigated. Considering those results, the karyological types of the salticid spiders are classified into 2 groups. One group containing the major species of this family has the chromosome number of $n=15$ and the sex-mechanism of the XXO-type. The other group is composed of the species of the genus *Myrmarachne*; the chromosome numbers are $n=12$ (11 autosomes+1 sex-chromosome) in 4 species of *M. formicaria, M. laetus, M. providens* and *M. rumunni*, and the chromosome number of *M. japonica* is $n=15$ (13 autosomes+2 sex-chromosomes) (HACKMAN, 1948; SUZUKI, 1954; BOLE-GOWDA, 1958). It is an interesting subject in view of karyology and systemactis of the salticid spiders that *M. japonica* shows such a remarkable case as an exception even to the other exceptions in this family. *Hasarius doenitzi* belongs to the former group that is composed of the great majority of the salticids.

Finally, is it concluded that the technique demonstrated in the present paper can be used for observation of most spider chromosomes. This technique using spider-eggs should be especially useful for the chromosomal preparations of such spiders that have too small gonads to be made into chromosomal preparations by the squash method, e.g., males of the spiders in the genera *Arachnura, Cyrtarachne, Gasteracantha* and *Thomisus* and both sexes in the families Theridiidae and Micryphantidae.

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References

AMITAI, S., M. WYSOKI and E. SWIRSKI, 1969. A case of thelytoky in a phytoseiid mite (Acarina: Mesostigmata), with cytological studies. *Isr. J. Agric. Res.*, **19** (1): 49–52.

BOLE-GOWDA, B. N., 1958. A study of the chromosomes during meiosis in twenty-two species of Indian spiders. *Proc. Zool. Soc. Bengal,* **11**: 69–108.

HACKMAN, W., 1948. Chromosomenstudien an Araneen mit besonderer Berücksichtigung der Geschlechtschromosomen. *Acta Zool. Fennica,* **54**: 1–101.

MITTAL, O. P., 1964. Karyological studies on the Indian spiders. II. An analysis of the chromosomes during spermatogenesis in five species of spiders belonging to the family Salticidae. *Res. Bull.* (*N. S.*) *Panjab Univ.,* **15** (3/4): 315–326.

MITTAL, O.P., 1966a. Karyological studies on the Indian spiders. V. Chromosome cycle in three species of the family Clubionidae. *Caryologia,* **19** (4): 385–394.

————, 1966b. Karyological studies on the Indian spiders. VI. Chromosome number and sex-determining mechanism in the family Araneidae. *Res. Bull.* (*N. S.*) *Panjab Univ.*, **17** (3/4): 335–351.

PAINTER, T. S., 1914. Spermatogenesis in spiders. *Zool. Jahrb., Anat. Ontog. Tiere,* **38**: 509–576.

SUZUKI, S., 1954. Cytological studies in spiders. III. Studies on the chromosomes of fifty-seven species of spiders belonging to seventeen families, with general considerations on chromosomal evolution. *J. Sci. Hiroshima Univ.,* Ser. B, Div. 1, **15** (2): 23–136, 15 pls.

WYSOKI, M., 1968. A smear method for making permanent mounts of the metaphase chromosomes in eggs of phytoseiid mites (Acarina: Mesostigmata). *Contrib. Volcani Inst. Agric. Res.,* (1342-E): 119–122.

摘　　要

松本誠治（杏林大学医学部生物学教室，〒192 東京都八王子市宮下町476）：クモ卵を用いた体細胞染色体の観察.

クモの体細胞染色体の観察のために，標本作成法として乾燥法（Flame-dry 法）の適用を，また材料としてクモ卵の使用を試みた．その結果，染色体数の計数および染色体の大きさの観察について，よい結果が得られた．この方法は生殖巣を得ることが困難な種では，有用であると考えられる．使用したクモの染色体数は次の通りであった． ハツリグモ (*Acusilas coccineus*): $2n=24$ および 26，イヅツグモ (*Anyphaena pugil*): $2n=26$ および 28，デーニッツハエトリ (*Hasarius doenitzi*): $2n=28$ および 30，ウヅキコモリグモ (*Pardosa T-insignita*): $2n=28$ および 30.